

# Effects of supplemental $\beta$ -carotene, cigarette smoking, and alcohol consumption on serum carotenoids in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study<sup>1-3</sup>

Demetrius Albanes, Jarmo Virtamo, Philip R Taylor, Matti Rautalahti, Pirjo Pietinen, and Olli P Heinonen

**ABSTRACT** We determined whether serum carotenoid or retinol concentrations were altered by  $\beta$ -carotene supplementation in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study and whether such effects were modified by alcohol consumption or cigarette use. Participants in this substudy were 491 randomly selected men aged 58–76 y from the metropolitan Helsinki study center [237 receiving supplemental  $\beta$ -carotene (20 mg/d) and 254 not receiving such supplementation]. Dietary carotenoids, retinol, and alcohol, and serum  $\beta$ -carotene,  $\alpha$ -tocopherol, retinol, and cholesterol were assessed at baseline. After an average of 6.7 y of supplementation, serum was collected and carotenoid, retinol, and  $\alpha$ -tocopherol concentrations were determined by HPLC. Serum carotenoid fractions were highly correlated with each other ( $P \leq 0.0001$ ). Compared with the unsupplemented group, the  $\beta$ -carotene group had significantly higher serum concentrations of  $\beta$ -carotene (1483%),  $\alpha$ -carotene (145%), and  $\beta$ -cryptoxanthin (67%) ( $P \leq 0.0001$ ). Retinol concentrations were 6% higher ( $P = 0.03$ ) and lutein was 11% lower ( $P = 0.02$ ) in the supplemented group. Serum lycopene, zeaxanthin, and  $\alpha$ -tocopherol did not differ according to  $\beta$ -carotene-supplementation status. Although these  $\beta$ -carotene-group differences were not significantly altered by amount of alcohol consumption, higher consumption ( $> 12.9$  g/d, median) was related to lower (10–38%) concentrations of carotenoids, particularly  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin, in both the supplemented and unsupplemented groups. Smoking status did not significantly influence the supplementation-related differences in serum carotenoid and retinol values but concentrations of carotenoids were generally highest in participants who quit smoking while in the study and lowest in current smokers of  $\geq 20$  cigarettes/d. This study showed that serum concentrations of non- $\beta$ -carotene carotenoids are altered by long-term  $\beta$ -carotene supplementation and confirms the adverse effects of alcohol and cigarette smoking on serum carotenoids. *Am J Clin Nutr* 1997;66:366–72.

**KEY WORDS**  $\beta$ -carotene; supplementation; carotenoids;  $\alpha$ -carotene;  $\beta$ -cryptoxanthin; lutein; lycopene; zeaxanthin; retinol; alcohol; cigarettes; smoking; Beta-Carotene and Retinol Efficacy Trial; Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

## INTRODUCTION

In two large randomized placebo-controlled cancer-prevention trials, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (1, 2) and the Beta-Carotene and Retinol Efficacy Trial (CARET) (3), an increased incidence of lung cancer and total mortality was observed in cigarette smokers who received  $\beta$ -carotene supplementation. The ATBC Study, which involved placebo-controlled supplementation with 20 mg  $\beta$ -carotene/d (with or without 50 mg  $\alpha$ -tocopherol) for 5–8 y (average: 6.1 y) in older middle-aged cigarette smokers, was recently corroborated by CARET. CARET tested  $\beta$ -carotene in combination with retinyl palmitate for an average of 3.7 y in smokers and people who had been exposed to asbestos and found somewhat greater relative increases in the occurrence of lung cancer and total mortality.

One possible mechanism that has been proposed to explain these findings is alteration of carotenoid metabolism by high-dose  $\beta$ -carotene supplementation, ie, interference with absorption, distribution, metabolism, or utilization of one or more of the other (non- $\beta$ -carotene) carotenoids sufficient to perturb their biological functions. Previous studies have found various changes in blood carotenoid concentrations in response to primarily short-term supplementation with  $\beta$ -carotene (4–9), lutein (6), and canthaxanthin (7). It is not known whether similar alterations occurred in the ATBC Study. The suggested alcohol-related augmentation of the  $\beta$ -carotene-related effect on lung cancer in the ATBC Study and CARET trials (10, 11) and the fact that these trials had similar results in populations of smokers (1, 3) are additional considerations of possible relevance to the main findings and warrant further study.

We investigated whether serum carotenoid or retinol concentrations were altered by supplementation with  $\beta$ -carotene in the ATBC Study, whether any such effects were modified by

<sup>1</sup> From the National Cancer Institute, Bethesda, MD; the National Public Health Institute, Helsinki; and the University of Helsinki.

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<sup>3</sup> Address reprint requests to D Albanes, National Cancer Institute, Executive Plaza North-Suite 211, 9000 Rockville Pike, Bethesda, MD 20892.

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amount of alcohol consumption or cigarette use, and whether exposure to alcohol and cigarette smoking affected serum concentrations of carotenoids or retinol.

## SUBJECTS AND METHODS

### Subjects

This study was conducted within the ATBC Study, a large randomized double-blind placebo-controlled prevention trial evaluating the effects of daily oral supplementation with  $\beta$ -carotene or  $\alpha$ -tocopherol on the incidence of lung and other cancers in male cigarette smokers. Study design, characteristics of participants, and trial results were described elsewhere (1, 2, 12). Participants in the current substudy ( $n = 491$ ) were subjects in the ATBC Study selected randomly from the metropolitan Helsinki study area during an 8-wk period in early 1993. At entry into the ATBC Study between 1985 and 1988, these men were aged 50–69 y, smoked  $\geq 5$  cigarettes/d, and were not taking daily supplements of vitamin E ( $> 20$  mg), vitamin A ( $> 20\,000$  IU), or  $\beta$ -carotene ( $> 6$  mg).

The subjects were randomly assigned to four study groups on the basis of a  $2 \times 2$  factorial design: participants received either  $\beta$ -carotene alone (20 mg *all-trans*- $\beta$ -carotene/d in 10% water-soluble beadlets;  $n = 110$ ), vitamin E alone (50 mg *dl*- $\alpha$ -tocopheryl acetate/d as a 50% powder;  $n = 128$ ), both agents ( $n = 127$ ), or placebo ( $n = 126$ ). Thus, 237 men were given  $\beta$ -carotene and 254 men were not given  $\beta$ -carotene. Capsules were provided by Hoffmann-La Roche Ltd, Basel, Switzerland. At the time of the follow-up blood collection in 1993, participants were aged 58–76 y (median: 66 y) and had been in the study and taking capsules for an average of 6.7 y.

### Baseline data

As part of the ATBC Study protocol for baseline data collection, tobacco use and other factors were recorded, height and weight were measured, and a serum sample was obtained (12). Serum concentrations of  $\beta$ -carotene,  $\alpha$ -tocopherol, and retinol were determined by HPLC (13) and serum total and high-density-lipoprotein cholesterol were analyzed enzymatically (12). Serum concentrations of carotenoids other than  $\beta$ -carotene were not determined at baseline. Tobacco use was recorded throughout the ATBC Study and 113 participants in this substudy (23%) reported having quit smoking cigarettes by the time of the follow-up serum collection early in 1993.

Dietary data were obtained for 467 subjects from a detailed food-use questionnaire that included a color picture booklet and questions on the usual frequency of consumption and portion sizes of 276 common foods, mixed dishes, and beverages (including alcohol) over the preceding 12 mo (14). Intake of carotenoids was based on Finnish food-composition data (15–18). By design of the parent study, dietary and alcohol data were collected during follow-up in a random sample (11%) of participants. Therefore, these data were not used in the analysis.

### Serum carotenoids during supplementation

For this study, 1 mL serum was obtained from each participant during his final ATBC Study follow-up visit to the study center in early 1993 and stored at  $-20^\circ\text{C}$  for an average of 14 mo. The assays for retinol, carotenoids, and  $\alpha$ -tocopherol were

performed in the analytic laboratories of the Vitamin Research Department of Hoffmann-La Roche Ltd (19, 20). Detection limits were as follows: lycopene,  $0.009\ \mu\text{mol/L}$ ;  $\beta$ -cryptoxanthin,  $0.018\ \mu\text{mol/L}$ ;  $\beta$ -carotene and  $\alpha$ -carotene,  $0.019\ \mu\text{mol/L}$ ; lutein and zeaxanthin,  $0.035\ \mu\text{mol/L}$ ; retinol,  $0.07\ \mu\text{mol/L}$ ; and  $\alpha$ -tocopherol,  $0.12\ \mu\text{mol/L}$ . CVs for multiple determinations in the same serum sample on the same day or different days were 2–10%.

All laboratory assays were conducted by persons who were blinded to intervention assignment and questionnaire data except during a later recheck of selected samples to exclude analytic artifact as the reason for the apparently increased  $\alpha$ -carotene values in the  $\beta$ -carotene group. This analysis showed a high selectivity and specificity for both  $\alpha$ -carotene and  $\beta$ -carotene values.

### Statistical analysis

Means  $\pm$  SDs were calculated for participant characteristics at baseline in the  $\beta$ -carotene and no- $\beta$ -carotene groups and  $t$  tests were performed. For on-study serum carotenoids, retinol, and  $\alpha$ -tocopherol values (some of which were skewed), medians and interquartile ranges were calculated for the  $\beta$ -carotene and no- $\beta$ -carotene groups overall and within subgroups created on the basis of daily baseline alcohol consumption (low or high compared with the median value) and number of cigarettes smoked daily at the time of the follow-up serum collection [none (ie, participant quit smoking during the study), 5–19, or  $\geq 20$ ].

Wilcoxon rank-sum tests were used to assess differences between groups and Spearman rank correlation coefficients between the carotenoids were calculated. General linear-regression models using both untransformed and log-transformed serum carotenoid values were used to adjust serum carotenoid concentrations simultaneously for several associated factors, including  $\alpha$ -tocopherol intervention group, dietary carotenoids and fat, serum cholesterol, body mass index (BMI), number of cigarettes, and alcohol consumption (21). The interaction between  $\beta$ -carotene-supplementation group and alcohol or smoking categories (as scored variables) was tested by adding a cross-product variable ( $\beta$ -carotene group  $\times$  alcohol or  $\beta$ -carotene group  $\times$  smoking category) in the regression models for each serum fraction. Analyses were done with SAS statistical software (SAS Institute Inc, Cary NC).

## RESULTS

The no- $\beta$ -carotene and  $\beta$ -carotene intervention groups were similar with respect to all baseline characteristics studied, including carotenoid intake (Table 1). These and other baseline characteristics were comparable with those in the overall ATBC Study population (12). There were no intervention-group differences in some of the same factors during supplementation (ie, number of cigarettes, serum cholesterol concentration, and BMI) or in compliance with taking capsules (median: 98.0% in both groups). At the time of serum collection for this study the men were aged 58–76 y (median: 66 y). A similar proportion of men in the  $\beta$ -carotene and the no- $\beta$ -carotene groups were assigned to the  $\alpha$ -tocopherol arm of the ATBC Study (54% and 50%, respectively).

TABLE 1

Characteristics of participants at study entry<sup>1</sup>

	No- $\beta$ -carotene group ( <i>n</i> = 254)	$\beta$ -Carotene group ( <i>n</i> = 237)
Age (y)		
Cigarettes/d		
Time smoking (y)		
Body weight (kg)		
BMI (kg/m <sup>2</sup> )		
Serum cholesterol (mmol/L)		
Serum retinol ( $\mu$ mol/L)		
Daily nutrient intake		
Energy (MJ)	10.77 $\pm$ 2.78	11.02 $\pm$ 2.56
Fat (g)	112.2 $\pm$ 35.0	115.2 $\pm$ 32.1
Cholesterol (mg)	518.4 $\pm$ 203.0	534.3 $\pm$ 203.0
Alcohol (g)	20.0 $\pm$ 21.0	18.1 $\pm$ 19.4
Carotenoids ( $\mu$ g)		
Total	4580 $\pm$ 2682	4514 $\pm$ 2372
$\beta$ -Carotene	2137 $\pm$ 1692	2135 $\pm$ 1562
Lutein/zeaxanthin	1378 $\pm$ 520	1403 $\pm$ 470
Lycopene	960 $\pm$ 904	875 $\pm$ 788
$\alpha$ -Carotene	101 $\pm$ 107	103 $\pm$ 100
Canthaxanthin	38 $\pm$ 52	39 $\pm$ 58
$\beta$ -Cryptoxanthin	3 $\pm$ 4	3 $\pm$ 3
Retinol ( $\mu$ g)	1734 $\pm$ 1288	1915 $\pm$ 1567
Vitamin E (mg)	12.0 $\pm$ 6.0	13.0 $\pm$ 6.0
Selenium ( $\mu$ g)	80.4 $\pm$ 24.0	84.0 $\pm$ 23.0
Vitamin C (mg)	104.1 $\pm$ 52.0	103.1 $\pm$ 49.0
Folate ( $\mu$ g)	309.0 $\pm$ 102.0	321.0 $\pm$ 91.0

<sup>1</sup>  $\bar{x} \pm$  SD. There were no significant differences between groups.

During supplementation the  $\beta$ -carotene group had substantially higher serum concentrations of not only  $\beta$ -carotene but also of  $\alpha$ -carotene and  $\beta$ -cryptoxanthin compared with the no- $\beta$ -carotene group (Table 2). Retinol concentrations were also slightly but significantly elevated. In contrast, the  $\beta$ -carotene group had lower serum lutein concentrations. There was no significant difference between the two groups in either serum  $\alpha$ -tocopherol or  $\alpha$ -tocopherol adjusted for cholesterol.

In the no- $\beta$ -carotene group, individual serum carotenoid fractions were significantly correlated with each other: correlation coefficients ( $r_s$ ) ranged from 0.20 for zeaxanthin and  $\beta$ -carotene (or lutein) ( $P \leq 0.002$ ) to 0.73 for  $\beta$ -carotene and

$\alpha$ -carotene ( $P \leq 0.0001$ ). Serum concentrations of specific carotenoids were also associated with intake of the respective carotenoids in the no- $\beta$ -carotene group ( $r_s = 0.37, 0.29, 0.21$ , and 0.18, respectively, for  $\alpha$ -carotene, lycopene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin,  $P \leq 0.0001$ ).  $\beta$ -Carotene was an exception ( $r_s = 0.05$ ). Serum  $\beta$ -carotene concentration was positively correlated with total fat intake in the  $\beta$ -carotene group ( $r_s = 0.31, P \leq 0.0001$ ) but not in the no- $\beta$ -carotene group ( $r_s = 0.07, P = 0.30$ ) and most serum carotenoids were associated with serum cholesterol in both groups.

Serum carotenoid and retinol concentrations during intervention, according to baseline consumption of alcohol and  $\beta$ -carotene-supplementation group, are shown in Table 3. There was a similar pattern of serum carotenoid and retinol differences in response to supplementation in the low- and high-alcohol-consumption categories, with significantly higher serum concentrations of  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin in the  $\beta$ -carotene group for each category of alcohol consumption. The interaction between  $\beta$ -carotene supplementation and alcohol in linear-regression models was not significant for serum carotenoids and retinol (both untransformed and log-transformed serum values), with the exception of a marginally significant ( $P = 0.08$ ) interaction for log-transformed serum  $\beta$ -carotene.

Higher alcohol consumption was generally associated with lower serum carotenoid concentrations and higher retinol concentrations in both the no- $\beta$ -carotene and  $\beta$ -carotene groups. These relations were significant by Wilcoxon rank-sum tests for  $\beta$ -carotene ( $P \leq 0.0001$  and  $P = 0.02$ , respectively, in the no- $\beta$ -carotene and  $\beta$ -carotene groups),  $\alpha$ -carotene ( $P = 0.002$  and  $P = 0.009$ ), and  $\beta$ -cryptoxanthin ( $P = 0.05$  and  $P = 0.009$ ). Simultaneous adjustment for  $\alpha$ -tocopherol-supplementation group, dietary carotenoid or retinol intake, dietary fat, serum cholesterol, number of cigarettes, and BMI by means of multivariate linear-regression modeling of untransformed or log-transformed values for serum carotenoids did not alter these differences. Use of the overall ATBC Study median for alcohol consumption (11 g ethanol/d) to define low- and high-consumption subgroups produced similar results.

Serum carotenoid and retinol concentrations according to smoking status at the time of follow-up blood sampling and  $\beta$ -carotene supplementation group are shown in Table 4.

TABLE 2

Serum carotenoids, retinol, and  $\alpha$ -tocopherol during the study

	No- $\beta$ -carotene group ( <i>n</i> = 254)	$\beta$ -Carotene group ( <i>n</i> = 237)	Difference <sup>1</sup>
$\beta$ -Carotene ( $\mu$ mol/L)	0.37 (0.21–0.58) <sup>2</sup>	5.81 (3.65–8.53)	
$\beta$ -Cryptoxanthin ( $\mu$ mol/L)	0.12 (0.06–0.25)	0.20 (0.11–0.33)	
Lycopene ( $\mu$ mol/L)	0.11 (0.04–0.22)	0.11 (0.06–0.20)	
$\alpha$ -Carotene ( $\mu$ mol/L)	0.06 (0.03–0.11)	0.15 (0.10–0.20)	
Lutein ( $\mu$ mol/L)	0.17 (0.12–0.22)	0.15 (0.12–0.20)	
Zeaxanthin ( $\mu$ mol/L) <sup>3</sup>	< 0.035 (< 0.035–0.040)	< 0.035 (< 0.035–0.040)	
Retinol ( $\mu$ mol/L)	1.94 (1.69–2.30)	2.05 (1.73–2.42)	
$\alpha$ -Tocopherol ( $\mu$ mol/L)	37.2 (30.0–44.8)	36.0 (29.5–44.8)	

<sup>1</sup> Based on values before truncation.<sup>2</sup> Median; 25–75 percentile in parentheses.<sup>3,4,6</sup> Significant difference between groups by Wilcoxon rank-sum test: <sup>3</sup>  $P \leq 0.0001$ , <sup>4</sup>  $P = 0.02$ , <sup>6</sup>  $P = 0.03$ .<sup>5</sup> Zeaxanthin concentrations below detectable limits are represented by < 0.035  $\mu$ mol/L.

TABLE 3

Serum carotenoids and retinol during the study, according to baseline alcohol consumption and supplementation group

	Low alcohol consumption ( $\leq 12.9$ g/d)			High alcohol consumption ( $> 12.9$ g/d)		
	No-β-carotene group ( <i>n</i> = 117)	β-Carotene group ( <i>n</i> = 116)	Difference <sup>1</sup>	No-β-carotene group ( <i>n</i> = 125)	β-Carotene group ( <i>n</i> = 109)	Difference
			%			
β-Carotene (μmol/L)	0.46 (0.29–0.64) <sup>2</sup>	6.54 (4.39–9.53)	1321 <sup>3</sup>	0.29 (0.16–0.49)	5.71 (3.12–7.92)	
β-Cryptoxanthin (μmol/L)	0.13 (0.07–0.33)	0.23 (0.14–0.40)	70 <sup>4</sup>	0.11 (0.06–0.21)	0.18 (0.10–0.28)	
Lycopene (μmol/L)	0.12 (0.04–0.24)	0.12 (0.07–0.21)	2	0.10 (0.05–0.18)	0.11 (0.06–0.18)	
α-Carotene (μmol/L)	0.08 (0.04–0.15)	0.16 (0.11–0.21)	111 <sup>3</sup>	0.05 (0.02–0.10)	0.14 (0.09–0.18)	
Lutein (μmol/L)	0.18 (0.13–0.24)	0.16 (0.12–0.21)	–13	0.17 (0.12–0.21)	0.14 (0.12–0.19)	
Zeaxanthin (μmol/L) <sup>6</sup>	< 0.035 (< 0.035–0.039)	< 0.035 (< 0.035–0.042)	0	< 0.035 (< 0.035–0.037)	< 0.035 (< 0.035–0.039)	
Retinol (μmol/L)	1.89 (1.68–2.22)	2.03 (1.69–2.38)	7	1.99 (1.73–2.38)	2.14 (1.85–2.50)	8

<sup>1</sup> Based on values before truncation.

<sup>2</sup> Median; 25–75 percentile in parentheses.

<sup>3–5</sup> Significant difference between supplementation groups by Wilcoxon rank-sum test: <sup>3</sup> *P* ≤ 0.0001, <sup>4</sup> *P* = 0.002, <sup>5</sup> *P* = 0.0004.

<sup>6</sup> Zeaxanthin concentrations below detectable limits are represented by < 0.035 μmol/L.

Smoking status did not materially influence the observed differences in serum carotenoid and retinol related to β-carotene supplementation and all tests of this interaction yielded non-significant results. Furthermore, with a few exceptions (eg, β-carotene), values for carotenoids were highest in participants who quit smoking during the study and lowest in current smokers of ≥ 20 cigarettes/d. Neither multivariate modeling to adjust for α-tocopherol supplementation, dietary carotenoid or retinol intake, dietary fat, number of cigarettes smoked at baseline, and BMI, nor use of baseline (instead of follow-up) smoking data, altered these findings.

## DISCUSSION

We observed higher serum concentrations of β-carotene, α-carotene, β-cryptoxanthin, and retinol and lower concentrations of lutein in participants who received β-carotene supplementation for nearly 7 y. Previous studies showed an expected although quantitatively variable rise in serum β-carotene resulting from β-carotene supplementation and fairly consistently found higher concentrations of α-carotene (4, 5, 9) and lower concentrations of lutein/zeaxanthin (4, 6, 8, 9). One study also showed increased β-cryptoxanthin in both men and women, lower lutein/zeaxanthin concentrations in men, and higher lutein/zeaxanthin concentrations in women (5). Three studies reported increased lycopene concentrations (5, 8, 9) and one study showed lower lycopene concentrations, probably as a result of the controlled diet used (4). Another study found diminished canthaxanthin concentrations from single-dose supplementation with a combination of β-carotene and canthaxanthin (6). The magnitude of the carotenoid changes was comparable if the dosages of β-carotene are taken into account. The data presented here provide clear evidence that long-term supplementation with β-carotene affected serum concentrations of other carotenoids and possibly retinol in participants in the ATBC Study.

How serum concentrations of other carotenoids are altered by β-carotene supplementation is unclear. Possible analytic artifacts from HPLC and contamination of the supplement with other carotenoids have been mentioned in some previous reports (4, 6) but were ruled out in this study. Supplemental β-carotene could influence non-β-carotene fractions through

modification of their absorption, distribution, storage, utilization, or clearance. For example, absorption of the other fractions might be enhanced during β-carotene supplementation. Metabolic substitution by β-carotene for biological (including antioxidant) functions of other carotenoids, such as greater conversion of the abundant β-carotene to vitamin A, with diminished conversion of either α-carotene or β-cryptoxanthin, is another hypothetical mechanism that might account for the serum or plasma changes. Alternatively, β-carotene might induce release of other carotenoids from specific tissues into serum. Because of the similar molecular structure of β-carotene, α-carotene, and β-cryptoxanthin, metabolic conversion of β-carotene to α-carotene or β-cryptoxanthin could also theoretically occur. However, only limited data exist to support such in vivo bioconversion of lutein, zeaxanthin, and metabolites, which involves migration of one double bond (22). Clearly, our knowledge about carotenoid metabolism is less than complete although continually expanding and further study of the observed carotenoid changes is needed.

Although early studies suggested that serum α-tocopherol was attenuated by β-carotene supplementation (23, 24), our data are in accord with the preponderance of findings from several large studies that showed no such attenuation (9, 25–29).

Our findings also raise the possibility, albeit remote, that one or more of the other serum components (or their altered distribution) was responsible for the untoward effects in the ATBC Study by means of either a direct harmful effect of a moiety that was increased (eg, α-carotene, β-cryptoxanthin, or retinol) or a decrease in a fraction (eg, lutein) that may be protective. We are not aware of any data from studies in humans that suggest the existence of harmful effects from other carotenoids aside from reports of reversible retinal crystallization resulting from canthaxanthin supplementation (30). In comparison, retinol (or related compounds) has been postulated to promote carcinogenesis and alcohol is thought to potentiate such an effect in certain organs (31).

Although the biochemical basis for metabolic conversion of β-carotene to retinol and its congeners is well established (32) and it is believed that under normal conditions, mechanisms that affect β-carotene absorption and metabolism can maintain homeostatic control of retinol concentrations in blood and other

TABLE 4

Serum carotenoids and retinol during the study, according to smoking status during study and supplementation group

	No- $\beta$ -carotene group	$\beta$ -Carotene group	Difference <sup>1</sup>
Quit smoking ( <i>n</i> = 54 no- $\beta$ -carotene, <i>n</i> = 59 $\beta$ -carotene)			
$\beta$ -Carotene ( $\mu\text{mol/L}$ )	0.43 (0.23–0.63) <sup>2</sup>	5.74 (4.46–7.92)	1245 <sup>3</sup>
$\beta$ -Cryptoxanthin ( $\mu\text{mol/L}$ )	0.17 (0.08–0.34)	0.26 (0.19–0.41)	53 <sup>4</sup>
Lycopene ( $\mu\text{mol/L}$ )	0.14 (0.06–0.25)	0.13 (0.07–0.24)	–9
$\alpha$ -Carotene ( $\mu\text{mol/L}$ )	0.67 (0.30–1.32)	1.66 (1.29–2.12)	148 <sup>3</sup>
Lutein ( $\mu\text{mol/L}$ )	0.18 (0.14–0.23)	0.17 (0.14–0.22)	–3
Zeaxanthin ( $\mu\text{mol/L}$ ) <sup>5</sup>	< 0.035	< 0.035	0 <sup>6</sup>
	(< 0.035–0.037)	(< 0.035–0.046)	
Retinol ( $\mu\text{mol/L}$ )	1.95 (1.73–2.27)	2.18 (1.82–2.46)	12
Smoking < 20 cigarettes/d ( <i>n</i> = 95 no- $\beta$ -carotene, 86 $\beta$ -carotene)			
$\beta$ -Carotene ( $\mu\text{mol/L}$ )	0.39 (0.21–0.61)	6.61 (4.07–9.52)	1603 <sup>3</sup>
$\beta$ -Cryptoxanthin ( $\mu\text{mol/L}$ )	0.13 (0.07–0.29)	0.20 (0.11–0.31)	50 <sup>7</sup>
Lycopene ( $\mu\text{mol/L}$ )	0.12 (0.04–0.22)	0.12 (0.06–0.20)	–3
$\alpha$ -Carotene ( $\mu\text{mol/L}$ )	0.75 (0.35–1.43)	1.40 (1.04–1.96)	87 <sup>3</sup>
Lutein ( $\mu\text{mol/L}$ )	0.18 (0.13–0.23)	0.15 (0.12–0.19)	16 <sup>7</sup>
Zeaxanthin ( $\mu\text{mol/L}$ ) <sup>5</sup>	< 0.035	< 0.035	0
	(< 0.035–0.042)	(< 0.035–0.040)	
Retinol ( $\mu\text{mol/L}$ )	1.94 (1.66–2.24)	2.00 (1.69–2.42)	3
Smoking $\geq$ 20 cigarettes/d ( <i>n</i> = 105 no- $\beta$ -carotene, <i>n</i> = 92 $\beta$ -carotene)			
$\beta$ -Carotene ( $\mu\text{mol/L}$ )	0.32 (0.17–0.52)	5.11 (3.34–8.10)	1500 <sup>3</sup>
$\beta$ -Cryptoxanthin ( $\mu\text{mol/L}$ )	0.10 (0.06–0.19)	0.15 (0.08–0.27)	41 <sup>7</sup>
Lycopene ( $\mu\text{mol/L}$ )	0.10 (0.04–0.16)	0.11 (0.06–0.18)	7
$\alpha$ -Carotene ( $\mu\text{mol/L}$ )	0.52 (0.28–0.82)	1.40 (0.89–1.90)	169 <sup>3</sup>
Lutein ( $\mu\text{mol/L}$ )	0.15 (0.11–0.21)	0.14 (0.11–0.20)	–6
Zeaxanthin ( $\mu\text{mol/L}$ ) <sup>5</sup>	< 0.035	< 0.035	0
	(< 0.035–0.035)	(< 0.035–0.037)	
Retinol ( $\mu\text{mol/L}$ )	1.94 (1.71–2.35)	2.02 (1.71–2.31)	4

<sup>1</sup> Based on values before truncation.<sup>2</sup> Median; 25–75 percentile in parentheses.<sup>3,4,6,7</sup> Significant difference between supplementation groups by Wilcoxon rank-sum test: <sup>3</sup> $P \leq 0.0001$ , <sup>4</sup> $P = 0.002$ , <sup>6</sup> $P = 0.04$ , <sup>7</sup> $P = 0.01$ <sup>5</sup> Zeaxanthin concentrations below detectable limits are represented by < 0.035  $\mu\text{mol/L}$ .

tissues, it is less clear whether long-term supplementation with  $\beta$ -carotene can measurably affect retinol concentrations in humans. Most  $\beta$ -carotene-supplementation studies did not measure or report data for retinol (4, 5–7, 9). Of the few that did, most found no changes in plasma or serum retinol in response to pharmacologic doses of  $\beta$ -carotene (9, 27, 28, 33, 34). It is of interest, however, that somewhat higher adipose tissue concentrations of retinoic acid were observed in subjects given a large single oral dose of  $\beta$ -carotene in one investigation (34) and that accumulation of vitamin A in the livers of preruminant calves fed higher dosages of  $\beta$ -carotene has been reported recently (35).

The alteration of serum carotenoids and retinol by  $\beta$ -carotene supplementation was not related to amount of alcohol consumption. This finding is evidence against the relevance of serum carotenoids and retinol to the reported interaction between  $\beta$ -carotene and alcohol in the incidence of lung cancer in the ATBC Study and CARET (10, 11). Our observation of lower serum carotenoid concentrations in men consuming more alcohol, a finding that was not accounted for by dietary carotenoid values, is consistent with most previous research, including cross-sectional (36–41) and  $\beta$ -carotene-supplementation studies (28, 33, 40). In contrast, one study found higher serum  $\beta$ -carotene in drinkers than in nondrinkers (42) and another found that plasma  $\beta$ -carotene and  $\alpha$ -carotene increased and  $\beta$ -cryptoxanthin and lutein/zeaxanthin decreased during

the alcohol-intake phase of a controlled dietary study (43). Given that several studies, including the present investigation, eliminated dietary differences as the reason for alcohol-related alterations in plasma or serum carotenoids (28, 33, 37, 41), more direct effects of alcohol on  $\beta$ -carotene and carotenoid absorption, metabolism, or utilization should be considered. Such effects are greater oxidative stress from alcohol leading to greater  $\beta$ -carotene (and other antioxidant) use by alcohol-related radicals (39, 42) and increased carotenoid conversion to retinol (41). In the current study, serum retinol concentrations were somewhat elevated in men with higher alcohol intake, regardless of  $\beta$ -carotene supplementation.

Our data also highlight the important intercorrelations among the various serum carotenoids and are consistent with the possibility that elevated serum  $\beta$ -carotene in previous observational studies may have served as an indirect marker of other carotenoids. Some recent observational data support a role for other specific carotenoids in human cancer (44, 45).

The strengths of this investigation include its large sample size, its randomized double-blind design, and the high-quality prospective dietary and alcohol data available for study. The supplementation period was 5–8 y (average: 7 y), which afforded more than adequate time for stabilization of absorption, metabolism, and clearance mechanisms relevant to carotenoids. Compliance in taking capsules was high. Serum samples were stored for an average of only 14 mo at  $-20^{\circ}\text{C}$  before analysis.



However, by design of the parent ATBC Study, only serum  $\beta$ -carotene was determined at study entry; thus, data on pre-supplementation and postsupplementation changes in the other serum fractions were not available. Also, because all subjects were cigarette smokers at study entry, lifelong nonsmokers were not studied.

In summary,  $\beta$ -carotene supplementation (20 mg/d) led to higher serum concentrations of  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, and retinol and modestly decreased lutein concentrations. Thus, our study did not support the notion that supplemental  $\beta$ -carotene suppresses carotenoid fractions other than lutein. Independent of dietary carotenoid intake, greater alcohol consumption and, to a lesser degree, current heavier cigarette smoking were related to lower serum carotenoid concentrations; however, the response of serum carotenoids to  $\beta$ -carotene supplementation was not related to the level of alcohol consumption or cigarette smoking. Studies aimed at determining how these effects occur and their relevance to trial-based cancer findings would be useful. ■

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## REFERENCES

1. The ATBC Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994;330:1029-35.
2. Albanes D, Heinonen OP, Huttunen JK, et al. Effects of  $\alpha$ -tocopherol and  $\beta$ -carotene supplements on cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Clin Nutr* 1995;62(suppl 6):1427S-30S.
3. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of the combination of beta-carotene and vitamin A on lung cancer incidence, total mortality, and cardiovascular mortality in smokers and asbestos-exposed workers. *N Engl J Med* 1996;334:1150-5.
4. Micozzi MS, Brown ED, Edwards BK, et al. Plasma carotenoid response to chronic intake of selected foods and  $\beta$ -carotene supplements in men. *Am J Clin Nutr* 1992;55:1120-5.
5. Wahlqvist M, Wattanapenpaiboon N, Macrae FA, Lambert JR, MacLennan R, Hsu-Hage BN. Changes in serum carotenoids in subjects with colorectal adenomas after 24 mo of  $\beta$ -carotene supplementation. Australian Polyp Prevention Investigators. *Am J Clin Nutr* 1994;60:936-43.
6. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and  $\beta$ -carotene when administered to human adults in separate or combined oral doses. *Am J Clin Nutr* 1995;62:604-10.
7. White WS, Stacewicz-Sapunzakis M, Erdman JW, Bowen PE. Pharmacokinetics of  $\beta$ -carotene and canthaxanthin after ingestion of individual and combined doses by human subjects. *J Am Coll Nutr* 1994;13:665-71.
8. Ribayo-Mercado JD, Ordovas JM, Russell RM. Effect of  $\beta$ -carotene supplementation on the concentrations and distribution of carotenoids, vitamin E, vitamin A, and cholesterol in plasma lipoprotein and non-lipoprotein fractions in healthy older women. *J Am Coll Nutr* 1995;14:614-20.
9. Fotouhi N, Meydani M, Santos M, Meydani SN, Hennekens CH, Gaziano JM. Carotenoid and tocopherol concentrations in plasma, peripheral blood mononuclear cells, and red blood cells after long-term  $\beta$ -carotene supplementation in men. *Am J Clin Nutr* 1996;63:553-8.
10. Albanes D, Heinonen OP, Taylor PR, et al.  $\alpha$ -Tocopherol and  $\beta$ -carotene supplements and lung cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study: effects of baseline characteristics and study compliance. *J Natl Cancer Inst* 1996;88:1560-70.
11. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer incidence and intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst* 1996;88:1550-9.
12. The ATBC Cancer Prevention Study Group. The alpha-tocopherol, beta-carotene lung cancer prevention study (ATBC Study): design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1994;4:1-10.
13. Milne DB, Botnen J. Retinol, alpha-tocopherol, lycopene, and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin Chem* 1986;32:874-6.
14. Pietinen P, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments. *Am J Epidemiol* 1988;128:655-66.
15. Heinonen M, Ollilainen V, Linkola E, Varo P, Koivisto P. Carotenoids and retinoids in Finnish foods: ready-to-eat foods. *J Food Compos Anal* 1988;1:221-30.
16. Heinonen M, Ollilainen V, Linkola E, Varo P, Koivisto P. Carotenoids and retinoids in Finnish foods: dietary fats. *J Food Compos Anal* 1988;1:334-40.
17. Heinonen M, Ollilainen V, Linkola E, Varo P, Koivisto P. Carotenoids and retinoids in Finnish foods: vegetables, fruits, and berries. *J Agric Food Chem* 1989;37:655-9.
18. Heinonen M, Ollilainen V, Linkola E, Varo P, Koivisto P. Carotenoids and retinoids in Finnish foods: cereal and bakery products. *Cereal Chem* 1989;66:270-7.
19. Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W. Simultaneous determination of retinol, tocopherols, carotenes, and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. *Int J Vitam Nutr Res* 1991;61:232-8.
20. Oberlin B, Hess D. Determination of zeaxanthin and lutein in plasma and liver with HPLC. Basel, Switzerland: Hoffmann-La Roche Ltd. (GCR 06-92357.)
21. SAS Institute Inc. SAS/STAT user's guide, version 6. 4th ed. Vols 1 and 2. Cary, NC: SAS Institute Inc, 1989.
22. Khachik F, Beecher GR, Smith JC Jr. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J Cell Biochem Suppl* 1995;22:236-46.
23. Mobarhan S, Bowen P, Andersen B, et al. Effects of  $\beta$ -carotene repletion on  $\beta$ -carotene absorption, lipid peroxidation, and neutrophil superoxide formation in young men. *Nutr Cancer* 1990;14:195-206.
24. Xu MJ, Plezia PM, Alberts DS, et al. Reduction in plasma or skin alpha-tocopherol concentration with long-term oral administration of beta-carotene in humans and mice. *J Natl Cancer Inst* 1992;84:1559-65.
25. Willett WC, Stampfer MJ, Underwood BA, Taylor JO, Hennekens CH. Vitamins A, E, and carotene: effects of supplementation on their plasma levels. *Am J Clin Nutr* 1983;38:559-66.
26. Nierenberg DW, Stukel TA, Baron JA, Dain BJ, Greenberg ER. Determinants of plasma levels of beta-carotene and retinol. The Skin Cancer Prevention Study Group. *Am J Epidemiol* 1989;130:511-21.
27. Albanes D, Virtamo J, Rautalahti M, et al. Serum beta-carotene before and after beta-carotene supplementation. *Eur J Clin Nutr* 1992;46:15-24.
28. Goodman GE, Metch BJ, Omenn GS. The effect of long-term  $\beta$ -carotene and vitamin A administration on serum concentrations of  $\alpha$ -tocopherol. *Cancer Epidemiol Biomarkers Prev* 1994;3:429-32.
29. Mobarhan S, Shiau A, Grande A, et al.  $\beta$ -Carotene supplementation results in an increased serum and colonic mucosal concentration of  $\beta$ -carotene and a decrease in  $\alpha$ -tocopherol concentration in patients with colonic neoplasia. *Cancer Epidemiol Biomarkers Prev* 1994;3:501-5.
30. Arden GB, Barker FM. Canthaxanthin and the eye: a critical ocular toxicologic assessment. *J Toxicol Cut Ocular Toxicol* 1991;10:115-55.
31. Mayne ST, Graham S, Zheng T. Dietary retinol: prevention or pro-

- motion of carcinogenesis in humans? *Cancer Causes Control* 1991;2:443-50.
32. Olson JA. Vitamin A, retinoids and carotenoids. In: Shils ME, Olson JA, Shike M, eds. *Modern nutrition in health and disease*. 8th ed. Philadelphia: Lea & Febiger 1993:287-307.
  33. Costantino JP, Kuller LH, Begg L, Redmond CK, Bates MW. Serum level changes after administration of a pharmacologic dose of beta-carotene. *Am J Clin Nutr* 1988;48:1277-83.
  34. Johnson EJ, Suter PM, Sahyoun N, Ribayo-Mercado JD, Russell RM. Relation between  $\beta$ -carotene intake and plasma and adipose tissue concentrations of carotenoids and retinoids. *Am J Clin Nutr* 1995;62:598-603.
  35. Hoppe PP, Chew BP, Safer A, Stegemann I, Biesalski HK. Dietary  $\beta$ -carotene elevates plasma steady-state and tissue concentrations of  $\beta$ -carotene and enhances vitamin A balance in preruminant calves. *J Nutr* 1996;126:202-8.
  36. Russel-Briefel R, Bates MW, Kuller LH. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. *Am J Epidemiol* 1985;122:741-9.
  37. Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am J Epidemiol* 1988;127:283-96.
  38. Ward RJ, Peters TJ. The antioxidant status of patients with either alcohol-induced liver damage or myopathy. *Alcohol Alcohol* 1992;27:359-65.
  39. Simonetti P, Cestaro B, Porrini M, Viani P, Roggi C, Testonli G. Effect of alcohol intake on lipids and fat-soluble vitamins in blood. *Minerva Med* 1993;84:447-52.
  40. Ahmed S, Leo MA, Lieber CS. Interactions between alcohol and  $\beta$ -carotene in patients with alcoholic liver disease. *Am J Clin Nutr* 1994;60:430-6.
  41. Lecomte E, Herbeth B, Pirollet P, et al. Effect of alcohol consumption on blood antioxidant nutrients and oxidative stress indicators. *Am J Clin Nutr* 1994;60:255-61.
  42. Simonetti P, Brusamolino A, Pellegrini N, et al. Evaluation of the effect of alcohol consumption on erythrocyte lipids and vitamins in a healthy population. *Alcohol Clin Exp Res* 1995;19:517-22.
  43. Forman MR, Beecher GR, Lanza E, et al. Effect of alcohol consumption on plasma carotenoid concentrations in premenopausal women: a controlled dietary study. *Am J Clin Nutr* 1995;62:131-5.
  44. LeMarchand L, Hankin JH, Kolonel LN, Beecher GR, Wilkens LR, Zhao LP. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1993;2:183-7.
  45. Ziegler RG, Colavito EA, Hartge P, et al. Importance of  $\alpha$ -carotene,  $\beta$ -carotene, and other phytochemicals in the etiology of lung cancer risk. *J Natl Cancer Inst* 1996;88:612-5.